PAT JT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 February 2001 (07.02.01)	
International application No. PCT/FI00/00220	**Applicant's or agent's file reference** 2990497PC/nu
International filing date (day/month/year) 17 March 2000 (17.03.00)	**Priority date** (day/month/year) 24 June 1999 (24.06.99)
Applicant HYÖTY, Heikki et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

10 January 2001 (10.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer R. E. Stoffel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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PCT

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WO 01/00236 A1

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(74) Agent: **KOLSTER OY AB**; Iso Roobertinkatu 23, P.O. Box 148, Fin-00121 Helsinki (FI).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AT (utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/00236 A1

(54) Title: PREVENTION OF TYPE 1 DIABETES AND OTHER NON-POLIO ENTEROVIRUS DISEASES

(57) Abstract: Live virus vaccines comprise attenuated viruses, while other vaccines comprise killed viruses or parts thereof. It has now been found that the immune response induced by oral poliovirus vaccine (OPV), which is a live vaccine, is cross-reactive with non-polio enteroviruses. OPV is therefore useful in the prevention of non-polio enterovirus diseases, especially Type 1 diabetes mellitus (IDDM). OPV is also useful in combination with killed/subunit non-polio enterovirus vaccines, whereby it prevents harmful side-effects of the killed/subunit vaccine by shifting the immune response from a harmful Th2- type response to a Th1 type response.

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WO 01/00236
PCT/FI00/00220

PATENT COOPERATION TREATY

15 -01- 2001

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
KOLSTER OY AB
Iso Roobertinkatu 23
P.O. Box 148
Fin-00121 Helsinki
FINLANDE

Date of mailing (day/month/year) 04 January 2001 (04.01.01)	
Applicant's or agent's file reference 2990497PC/nu	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/FI00/00220	17 March 2000 (17.03.00)	24 June 1999 (24.06.99)

Applicant
HYÖTY, Heikki et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AG,AU,DZ,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,
   NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   04 January 2001 (04.01.01) under No. WO 01/00236

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT IB 301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT IB 308 (July 1996)

EXPRESS MAIL LABEL NO.: EV 011019507 US

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To:

KOLSTER OY AB
Iso Roobertinkatu 23
P.O. Box 148
Fin-00121 Helsinki
FINLANDE

Date of mailing (day/month/year)
07 February 2001 (07.02.01)

Applicant's or agent's file reference
2990497PC/nu

IMPORTANT INFORMATION

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/FI00/00220	17 March 2000 (17.03.00)	24 June 1999 (24.06.99)

Applicant
HYÖTY, Heikki et al

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,CA,CN,CZ,DE,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US

2. The following Offices have waived the requirement for the notification of their election: the notification will be sent to them by the International Bureau only upon their request:

   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
   National :AE,AG,AL,AM,AT,AZ,BA,BB,BR,BY,CH,CR,CU,DK,DM,DZ,EE,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MW,MX,
   PT,SD,SG,SI,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
R. E. Stoffel

Telephone No. (41-22) 338.83.38

3820027

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70) 14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
2990497PC/or		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/FI00/00220	17/03/2000	24/06/1999
International Patent Classification (IPC) or national classification and IPC A61K39/125		
Applicant HYÖTY, Heikki et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10/01/2001	31.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel +49 89 2399 - 0 Tx: 523656 epmu d Fax +49 89 2399 - 4465	Authorized officer Favre, N Telephone No +49 89 2399 7363

Form PCT IPEA 409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/FI00/00220

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-26 as originally filed

Claims, No.:

1-35 as originally filed

Drawings, sheets:

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 19-33 and 35, with regard to industrial applicability.

because:

☒ the said international application, or the said claims Nos. 19-33 and 35 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1-11 and 14-35

	No:	Claims	12, 13
Inventive step (IS)	Yes:	Claims	1-11 and 16- 35
	No:	Claims	12-15
Industrial applicability (IA)	Yes:	Claims	1-18 and 34
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 19-33 and 35 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. For the assessment of the present claims 1-35 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognise as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2. The present invention relies on the surprising findings that in the Finnish population, the birth cohort which has been immunised by live poliovirus vaccine (OPV) has a significantly lower cumulative incidence of type 1 diabetes mellitus (IDDM) than the birth cohort that has been vaccinated with inactivated poliovirus vaccine (IPV) only (page 14, lines 14-36 and Figure 1). Moreover, the children whose mother had been vaccinated with OPV during pregnancy also have a significantly lower cumulative incidence of IDDM when compared to the children whose mother had been vaccinated with IPV during pregnancy.

2.1 Document D1 (Drug Safety, 1999, **20**(3):207-212) hypothesises that IDDM could be induced by coxsackie B-enterovirus (page 210, column 1, lines 17-36) and discloses (cf. abstract) that poliovirus vaccine (IPV) could protect against IDDM which is a non-polio enterovirus disease.

Document D2 (Journal of Medical Virology, 1998, **54**:226-232) strongly sustains this hypothesis by showing that there is a correlation between the cellular immune response (T-cell proliferation) to polioviruses (IPV) and to coxsackievirus B4 (page 227, column 2, lines 29-48).

Whereas D1 and D2 only refer to inactivated poliovirus vaccine (IPV = Salk vaccine), document D3 (American Family Physician, 1999, **59**(1):113-118; this document was not cited in the international search report) refers to the different poliovirus vaccine options (e.g. Table 1), comprising either IPV, oral poliovirus vaccine (OPV) and combination thereof.

2.2 However, neither D1, D2 or D3, nor any of the prior art documents cited discloses or fairly suggests the particular use of **OPV** for the manufacture of a vaccine against non-polio enterovirus diseases, e.g. IDDM, as defined in independent claim 1. Hence, the subject-matter of independent claim 1 is novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

2.3 Dependent claims 2-11 further define specific embodiments of the novel and inventive use of claim 1. Dependent claims 2-11 are thus also considered to meet the requirements of Articles 33(2) and 33(3) PCT.

3. Document D3 discloses (e.g. Table 1) vaccines comprising combinations of IPV and OPV. Document D2 discloses that IPV induces a cellular immune response (T-cell proliferation) to polioviruses and to non-polio enteroviruses (e.g. page 230, column 1, line 3 - column 2, line 19). The applicant argued that the vaccine of independent claim 12 differ from those of D3 in that they comprise an oral poliovirus vaccine and a **non**-polio enterovirus vaccine. This is however not reflected in the wording of the claim which leaves the nature of the second vaccine open, i.e. any vaccine, for example IPV (see also dependent claim 13).

Hence, the vaccines defined in claims 12 and 13 are not novel over the vaccines of D3.
Said claims 12 and 13 thus do not meet the requirements of Article 33(2) PCT.

3.1 The problem to be solved in claims 14 and 15 could be seen as the provision of a vaccine against non-polio enterovirus diseases.
As argued by the applicant, the disclosures of D1-D3 and the general knowledge in the art at the time was that enterovirus-based vaccines could lead to detrimental cross-reactivity, and for instance induce autoimmune diseases as IDDM. This is also illustrated by the examples of the present application (see Item VIII, point 2.). Thus it is agreed that the skilled person would not have been prompted to combine OPV with enterovirus antigens in view of the teachings of D2 and D3 (see above).
However, the application as filed fails to convincingly demonstrate that the vaccines defined in dependent claims 14 and 15 effectively protect against non-polio enterovirus diseases. In view of the above, there are thus substantive doubts whether the claimed vaccines solve the above-mentioned technical problem over its whole range.
Given these doubts, claims 14 and 15 are not considered to provide a solution to said problem over their whole range and thus cannot be considered as being inventive in the sense of Article 33(3) PCT.

4. The subject-matter of claims 16-18 does apparently not differ from that defined in independent claim 1, in view of the embodiments defined in dependent claims 11, 3, 6 and 7 (see also Item VIII, points 1. and 2.). Thus, in the light of the argumentation of points 2.-2.3 above, said claims 16-18 are also considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

5. The argumentation of points 2.-2.3 above also applies for the methods of preventing non-polio enterovirus diseases, e.g. IDDM, defined in claims 19-34 and 35 (see also Item VIII point 2.).
Hence, claims 19-34 and 35 meet the requirements of Articles 33(2) and 33(3) PCT.

6. Accordingly, the vaccine defined in claim 34 is also considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT (see also Item VIII point 2.).

Re Item VIII

Certain observations on the international application

1. Although claims 1, 2 and 16 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought and in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 1, 2 and 16 do not meet the requirements of Article 6 PCT.

1.1 Similarly, claims 19-23, 26, 31 and 35, which have been drafted as separate independent claims, appear to relate effectively to the same subject-matter. Said claims 19-23, 26, 31 and 35 thus do not meet the requirements of Article 6 PCT.

2. As presented in the description and emphasised by the applicant in its letter of reply, the present invention is based on the finding that OPV administration can protect against non-polio enterovirus diseases, e.g. IDDM. Furthermore, in the experimental section of the description, evidence is provided that IPV (not OPV) **worsen** the course of coxsackievirus B3 in a murine experimental model. Thus, it is clear from the description and from the arguments presented by the applicant that OPV is essential to the definition of the present invention.
Since independent claims 16, 31 and 34 do not contain this feature, they do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

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PCT REQUEST

2990497PC/nu

Original (for SUBMISSION) - printed on 17.03.2000 01:14:29 PM

0 0-1	For receiving Office use only International Application No.	PCT/FI 00/00220
0-2	International Filing Date	17 MAR 2000 (17.03.00)
0-3	Name of receiving Office and "PCT International Application"	The Finnish Patent Office PCT International Application
0-4 0-4-1	**Form - PCT/RO/101 PCT Request** Prepared using	PCT-EASY Version 2.90 (updated 08.03.2000)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	National Board of Patents and Registration (Finland) (RO/FI)
0-7	**Applicant's or agent's file reference**	2990497PC/nu
I	**Title of invention**	PREVENTION OF TYPE 1 DIABETES AND OTHER NON-POLIO ENTEROVIRUS DISEASES
II II-1 II-2 II-4 II-5 II-6 II-7	**Applicant** This person is: Applicant for Name (LAST, First) Address: State of nationality State of residence	 applicant and inventor all designated States HYÖTY, Heikki Minna Canthin katu 3 B FIN-33230 Tampere Finland FI FI
III-1 III-1-1 III-1-2 III-1-4 III-1-5 III-1-6 III-1-7	**Applicant and/or inventor** This person is: Applicant for Name (LAST, First) Address: State of nationality State of residence	 applicant and inventor all designated States KNIP, Mikael Palomäentie 11 A FIN-33230 Tampere Finland FI FI

EXPRESS MAIL LABEL
NO.: EV 011019507 US

PCT REQUEST

Original (for SUBMISSION) - printed on 17.03.2000 01:14:29 PM

IV-1	**Agent or common representative; or address for correspondence** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name	KOLSTER OY AB
IV-1-2	Address:	Iso Roobertinkatu 23 P.O. Box 148 FIN-00121 Helsinki Finland
IV-1-3	Telephone No.	358 9 618 821
IV-1-4	Facsimile No.	358 9 602 244
IV-1-5	e-mail	kolster@kolster.fi
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AG AL AM AT (patent and utility model) AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ (patent and utility model) DE (patent and utility model) DK (patent and utility model) DM DZ EE (patent and utility model) ES FI (patent and utility model) GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR (patent and utility model) KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK (patent and utility model) SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST

2990497PC/nu

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V-5	**Precautionary Designation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	24 June 1999 (24.06.1999)	
VI-1-2	Number	60/140,872	
VI-1-3	Country	US	
VII-1	**International Searching Authority Chosen**	European Patent Office (EPO) (ISA/EP)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	26	-
VIII-3	Claims	4	-
VIII-4	Abstract	1	2990497p.txt
VIII-5	Drawings	1	-
VIII-7	TOTAL	36	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-9	Separate signed power of attorney	✓	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-18	**Figure of the drawings which should accompany the abstract**	-	
VIII-19	**Language of filing of the international application**	English	
IX-1	**Signature of applicant or agent**	Tapio Valkeiskangas	
IX-1-1	Name	KOLSTER OY AB	

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10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**		

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11119804 97414199 PMID: 9269056

Influence of host related factors on the antibody response to trivalent oral polio vaccine in Tunisian infants.

Triki H; Abdallah M V; Ben Aissa R; Bouratbine A; Ben Ali Kacem M; Bouraoui S; Koubaa C; Zouari S; Mohsni E; Crainic R; Dellagi K

Institut Pasteur de Tunis, WHO Regional Reference Laboratory on Poliomyelitis, Belvedere, Tunisia.

Vaccine (ENGLAND) Jul 1997, 15 (10) p1123-9, ISSN 0264-410X
Journal Code: 8406899

Document type: Clinical Trial; Journal Article; Multicenter Study

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

The low efficiency of trivalent oral polio vaccine (TOPV) in inducing protective antibody titres to polio3 is a problem of great importance in many regions of the world. A prospective study was conducted in 121 Tunisian infants aged 3 months during routine immunization with TOPV under carefully controlled conditions. Seroconversion rates to polio1, polio2 and polio3, one month after the third dose, were 94.7, 100 and 89.5%, respectively. The kinetics of the antibody response showed delayed and more difficult responses to polio3 compared to polio2 and polio1. The following host related factors, previously suggested to interfere with the immune response, were assessed: maternal antibodies; breast-feeding; concurrent enteric infections; and other illnesses. The main factor associated with the lack of seroconversion was concurrent infection with non-polio enteroviruses (NPE) which was found in 50% of non-responders to polio1 and/or to polio3 during the vaccination protocol whereas no NPE was isolated in vaccine responders. The other studied factors seemed not to interfere in the infants according to the locally adopted vaccination schedule and to the specific socio-economic conditions.

Record Date Created: 19971020

3/7/23
DIALOG(R)File 155:MEDLINE(R)

08215483 94281424 PMID: 8011825
Oral polio vaccination during pregnancy: lack of impact on fetal development and perinatal outcome.
Harjulehto-Mervaala T; Aro T; Hiilesmaa V K; Hovi T; Saxen H; Saxen L
Department of Pathology, University of Helsinki, Finland.
Clinical infectious diseases - an official publication of the Infectious Diseases Society of America (UNITED STATES) Mar 1994, 18 (3) p414-20, ISSN 1058-4838 Journal Code: 9203213
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Prompted by a nascent epidemic of poliomyelitis in Finland, a mass vaccination program with live oral poliovirus vaccine (OPV) was implemented in February and March 1985. The final rate of coverage was approximately 94%. Pregnant women were included, and a cohort study was launched to evaluate any harmful effects of OPV on the developing embryo. All records of births to mothers who were pregnant during the period of vaccination and whose infants were delivered at the three major hospitals in the Helsinki area were reviewed. Within the study cohort, mothers were grouped into three categories according to their trimester of pregnancy during the program. In addition, two reference cohorts were evaluated; these cohorts consisted of infants delivered at the same hospitals during the second half of 1984 and of 1986, respectively. Each of the three categories in the study cohort included approximately 3,000 children, while each reference cohort included approximately 6,000 children. Data were analyzed on the rate of intrauterine growth and the prevalences of stillbirth, neonatal death, congenital malformation, premature birth, perinatal infection, and neurological aberration. No differences were documented among the study and reference cohorts or among the three categories within the study cohort. Thus, under the conditions described here, the inclusion of pregnant women in programs of mass vaccination with OPV appears to be safe.
Record Date Created: 19940727
Record Date Completed: 19940727
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3/7/4
DIALOG(R)File 155:MEDLINE(R)

11155256 98031083 PMID: 9364670
CPV vs IPV--could placental immunity reduce the number of vaccine-associated paralytic poliomyelitis?
Fescharek R; Budde R K; Arras C
Vaccine (ENGLAND) Nov 1997, 15 (16) p1707-8, ISSN 0264-410X
Journal Code: 8406899
Document type: Letter
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Record Date Created: 19980211
Record Date Completed: 19980211

8/7/73
DIALOG(R)File 155:MEDLINE(R)

07998362 94064079 PMID: 8244504
Oral polio vaccination in infants: beneficial effect of additional dose at birth.
Khare S; Kumari S; Nagpal I S; Sharma D; Verghese T
National Institute of Communicable Diseases, Delhi.
Indian journal of pediatrics (INDIA) Mar-Apr 1993, 60 (2) p275-81, ISSN 0019-5456 Journal Code: 0417442
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
This study was done to assess the response of newborns to trivalent oral polio vaccine and to study any efficacy of OPV if given to infants on third day of life. The study was conducted in two groups, A (87) and B (55) of infants in Delhi, India. In group A, the children received one birth dose or 'O' dose of TOPV, followed by 3 conventional doses started at 6 weeks, and in group B the children received only 3 doses of OPV. Pre and one month post immunization serum samples were tested for the presence of neutralising antibodies. In addition, in group A serum samples were collected at 6 weeks before the administration of 1st dose to see the sero response following 'O' dose of TOPV. It was found that administration of OPV on 3rd day of life leads to sero conversion in 15.3% of infants to all three polio virus types by the age of 6 weeks, and highest sero response was seen for polio virus type 1. Sero-conversion in group A was significantly more than sero-conversion in group B after the administration of last dose. Thus the study has established that immunization of newborns with TOPV is a safe and effective means for improving protection against the disease.
Record Date Created: 19940103
Record Date Completed: 19940103
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3/7/21
DIALOG(R)File 155:MEDLINE(R)

08562148 95250449 PMID: 7732798
Oral poliovirus vaccination and pregnancy complications.
Harjulehto-Mervaala T; Hovi T; Aro T; Saxen H; Hiilesmaa V K
Department of Pathology, University of Helsinki, Finland.
Acta obstetricia et gynecologica Scandinavica (DENMARK) Apr 1995, 74 (4) p262-5, ISSN 0001-6349 Journal Code: 0370343
Document type: Journal Article; Multicenter Study
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND. To determine whether the effect of live attenuated oral polio virus vaccine given to pregnant women increases pregnancy complications. METHODS. A study of women who had been vaccinated against poliovirus during a national vaccination campaign and who had delivered by cesarean section in three obstetrical hospitals in southern Finland. One thousand seven hundred and forty-seven vaccinated women (in three study cohorts), and their 2293 nonvaccinated controls (in two reference cohorts) were analyzed. Subjects are out of 22,000 deliveries evaluated earlier. RESULTS. Vaccinated sectioned women did not show an excess of pregnancy complications. The mean rate of cesarean sections was 18.4% in the study cohorts and 18.9% in the reference cohorts counted from the 22,000 deliveries. CONCLUSIONS. Oral live attenuated polio virus vaccine does not increase pregnancy complications and is considered a safe alternative for vaccinating pregnant women.
Record Date Created: 19950601
Record Date Completed: 19950601

t s4/7/14 18 19

4/7/14
DIALOG(R)File 155:MEDLINE(R)

10966819 97319506 PMID: 9176409
Coxsackievirus B3-induced myocarditis. Characterization of stable attenuated variants that protect against infection with the cardiovirulent wild-type strain.
Zhang H; Morgan-Capner P; Latif N; Pandolfino Y A; Fan W; Dunn M J; Archard L C
Department of Biochemistry, Charing Cross and Westminster Medical School, University of London, United Kingdom.
American journal of pathology (UNITED STATES) Jun 1997, 150 (6) p2197-207, ISSN 0002-9440 Journal Code: 0370502
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Coxsackievirus B3 (CVB3) is the enterovirus most frequently involved in human myocarditis or dilated cardiomyopathy. Attenuated variants were derived from a cardiovirulent CVB3 reactivated from a sequenced, full-length cDNA clone. The prophylactic potential of these variants was assessed in SWR/Ola (H-2q) mice. Animals immunized with attenuated variants of CVB3 were protected from myocarditis when challenged subsequently with the cardiovirulent wild-type virus. In contrast to nonimmunized controls, the wild-type virus was not isolated from myocardium of protected mice, nor was viral RNA detected in myocardium by reverse transcription nested polymerase chain reaction. Specific antibody to CVB3 was demonstrated by virus neutralization assay and by indirect immunofluorescence. The attenuated phenotype of one variant, p14V-1, remained stable throughout 20 consecutive passages in SWR mice and induced a markedly lower level of autoantibody against mouse cardiac myosin heavy chain than the cardiovirulent wild type. These data demonstrate that attenuated strains protect against CVB3-induced myocarditis in mice, that the attenuated phenotype is stable, and that they do not persist in myocardium nor induce a significant level of anti-heart anti-body against myosin heavy chain. These attenuants may be the basis of a live vaccine against CVB3 in the prevention of enteroviral heart muscle disease.
Record Date Created: 19970703
Record Date Completed: 19970703

? t s8/7/1 2

8/7/1
DIALOG(R)File 155:MEDLINE(R)

11027477 97381062 PMID: 9238419
[The latest on enteroviruses in human pathology]
Actualite des enterovirus en pathologie humaine.
Pozzetto B; Bourlet T
Laboratoire de bacteriologie-virologie, Faculte de medecine, J.-Lisfranc, Saint-Etienne, France.
Annales de biologie clinique (FRANCE) May-Jun 1997, 55 (3) p183-8, ISSN 0003-3898 Journal Code: 2984690R
Document type: Journal Article; Review; Review, Tutorial ; English Abstract
Languages: FRENCH
Main Citation Owner: NLM
Record type: Completed
Enteroviruses are small RNA viruses belonging to the Picornaviridae family. At least 65 serotypes have been described, including polioviruses, coxsackieviruses A and B, echoviruses and unclassified enteroviruses. Because of the absence of envelope they are relatively resistant to physical and chemical agents. They are mainly transmitted by the oral-fecal mode, but respiratory and mucosal transmissions are also possible. In humans, enteroviruses have been involved in miscellaneous acute infections and more recently in persistent infections (chronic meningoencephalitis in agammaglobulinemic patients, post-polio syndrome, chronic myocarditis and dilated cardiomyopathy, insulin-dependent diabetes mellitus...). Hypotheses in the relation between enterovirus persistence and chronic infections are formulated. The virological diagnosis of enterovirus infections is discussed, with a special focus on genomic application techniques (PCR) that are renewing the interest for this family of viruses in clinical pathology. If the role of enteroviruses in chronic pathologies is confirmed, the development of new therapeutic approaches (including vaccines and antiviral agents) will be needed. (43 Refs.)
Record Date Created: 19970821
Record Date Completed: 19970821

07841008 93296653 PMID: 8390713

High yield production of an inactivated coxsackie B3 adjuvant vaccine with protective effect against experimental myocarditis.

Fohlman J; Pauksen K; Merein B; Bjare U; Ilback N G; Friman G

Dept of Infectious Diseases, University Hospital, Uppsala, Sweden.

Scandinavian journal of infectious diseases. Supplementum (SWEDEN) 1993, 88 p103-8, ISSN 0300-8878 Journal Code: 0251025

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Dilated cardiomyopathy, perhaps chronic postviral fatigue syndrome as well as juvenile diabetes could be triggered by enteroviral infections. The frequency of sudden death after myocarditis and its relationship to enteroviral infections is disputed. Neonatal enteroviral disease is rare, but can be severe. It is also possible that enteroviruses pose a threat to immunocompromised patients, like bone marrow transplant recipients. Consequently, the emergence of chronic enteroviral diseases as a concept, prompted our attempts to produce an enteroviral vaccine. 1. Live attenuated enterovirus strains were previously in some cases shown to be suitable as vaccine candidates. We obtained neutralizing antibody titres ranging from 40-2560 against Coxsackie B3 virus (RD strain). Animals were protected to 90% against challenge infection. 2. Inactivated whole vaccine. We used beta-propiolactone to inactive Coxsackie B3 virus. 74% of the animals survived if the vaccine was prepared with Quil A matrix as adjuvant. The neutralisation antibody titres varied from < 5 to 320. By comparison aluminium hydroxide ($p = 0.06$) and Freund's adjuvant were inferior ($p < 0.01$). 3. Subunit vaccines. We have previously used the ISCOM (immunostimulatory complex) technology to produce a Coxsackie B3 subunit vaccine. High levels of neutralizing antibodies were obtained (512)-comparable to natural infection. All animals survived challenge infection after two booster doses with 16 nanogram of the ISCOM preparation. Limiting for this technique was the availability to include sufficient amount of antigenic protein material. In addition to neutralizing antibodies a cellular response might be obtainable. In conclusion we have shown that vaccine can be made against Coxsackie B3 virus with good protective effect and significant neutralisation antibody titre.(ABSTRACT TRUNCATED AT 250 WORDS)

Record Date Created: 19930720

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18/7/14
DIALOG(R)File 155:MEDLINE(R)

09219978 20530124 PMID: 11079740
Infections and risk of Type I (insulin-dependent) diabetes mellitus in Lithuanian children.
Pundziute-Lycka A; Urbonaite B; Dahlquist G
Department of Clinical Sciences, Paediatrics, Umea University, Sweden.
Diabetologia (GERMANY) Oct 2000, 43 (10) p1229-34, ISSN 0012-186X
Journal Code: 0006777
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
AIMS/HYPOTHESIS: The role of infections in the aetiology of Type I diabetes is controversial. Certain enteroviral infections might be involved in triggering the beta-cell destruction but insufficient exposure to early infections might increase the risk. We studied how the number of infections experienced during several periods from birth to onset influence diabetes risk. METHODS: The study group came from the five largest Lithuanian cities: 124 patients, selected from the 0-14 years-of-age childhood diabetes register and 372 population-based control subjects matched with them for age group and sex. Information about infections and duration of breastfeeding was collected from health care booklets, other data from a mailed questionnaire, returned by 94.4% of patients and 72.6% of control subjects. RESULTS: One or more infections experienced during the first half year of life tended to reduce diabetes risk. Crude odds ratios (95% confidence intervals) in the 0-14, 0-4 and 5-14 years-of-age groups were 0.66 (0.42-1.04), 1.06 (0.48-2.36) and 0.52 (0.30-0.90) respectively. Adjustment for the duration of breastfeeding, number of people in the household, duration of mother's education and birth order of the index child made little difference. Odds ratios (95% confidence intervals) in the 0-14, 0-4 and 5-14 years-of-age groups were 0.60 (0.37-0.98), 0.94 (0.40-2.20) and 0.47 (0.26-0.87), respectively. The number of infections recorded during the last pre-onset year or from birth to onset did not influence diabetes risk. CONCLUSION/INTERPRETATION: Exposure to infections early in life could decrease diabetes risk, particularly for children diagnosed after the age of 4 years.
Record Date Created: 20001128
Record Date Completed: 20010531

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10069130 22015002 PMID: 12021080
Viruses and diabetes.
Jaeckel Elmar; Manns Micheal; Von Herrath Matthias
Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute, Boston, Massachusetts 02115, USA. jaeckel elmar@yahoo.com
Annals of the New York Academy of Sciences (United States) Apr 2002, 958 p7-25, ISSN 0077-8923 Journal Code: 7506858
Document type: Journal Article; Review; Review, Academic
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Insulin-dependent diabetes mellitus (IDDM) is a multifactorial disease. Besides a genetic predisposition environmental factors have been implicated in the pathogenesis of beta cell destruction. Among these environmental factors viruses have been the focus of many studies. Some viruses are diabetogenic in animals, and others have been implicated as triggers in human IDDM by temporal and geographical association between IDDM and viral infections, serological evidence of infection in recently diagnosed diabetic patients, and the isolation of viruses from the pancreas of affected individuals. We discuss possible pathomechanisms of viral infections in beta cell destruction and review the studies on involvement of enteroviruses, retroviruses, rubella viruses, cytomegaloviruses, and Epstein-Barr viruses in human IDDM. We also report on studies of diabetogenic viruses in animal models as well as on viral infections protecting from IDDM. Some of the difficulties in linking viral infections to IDDM will be illustrated with data from a transgenic mouse model in which IDDM can be precipitated by infections with certain strains of lymphocytic choriomeningitis virus (LCMV). Emerging treatment concepts that do not rely on defining the initiating autoantigens but involve self-reactive regulatory lymphocytes such as oral antigen administration, as well as DNA vaccines, will be discussed briefly. (102 Refs.)
Record Date Created: 20020521
Record Date Completed: 20020712

/7/11
DIALOG(R)File 155:MEDLINE(R)

10861778 97213240 PMID: 9060905
Insulin-dependent diabetes mellitus: the hypothesis of molecular mimicry between islet cell antigens and microorganisms.
Maclaren N K; Atkinson M A
New Orleans Research Institute, New Orleans Children's Hospital, LA 70118, USA.
Molecular medicine today (ENGLAND) Feb 1997, 3 (2) p76-83, ISSN 1357-4310 Journal Code: 9508560
Contract/Grant No.: HD 19469-12; HD; NICHD
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Insulin-dependent diabetes mellitus (IDDM) in humans and the non-obese diabetic mouse is a polygenic disease, resulting from an autoimmune destruction of the insulin-secreting pancreatic beta cells. At least in NOD mice, the process is mediated through a T helper 1-cell-mediated cytotoxicity pathway. Although there is much circumstantial evidence to suggest that IDDM is environmentally induced, recent studies support the possibility that the inductive event involves cross-reactive immune responses to antigenic epitopes acting as molecular mimics between microbial proteins and autoantigens expressed by pancreatic insulin-secreting beta cells. The following article reviews the evidence for this concept. (37 Refs.)
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DIALOG(R)File 155:MEDLINE(R)

11464840 98348315 PMID: 9635154
Autoimmunity. The pathogen connection.
Benoist C; Mathis D
Nature (ENGLAND) Jul 16 1998, 394 (6690) p227-8, ISSN 0028-0836
Journal Code: 0410462
Document type: News
Languages: ENGLISH
Main Citation Owner: NLM
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(74) Agent: **KOLSTER OY AB**; Iso Roobertinkatu 23, P.O. Box 148, Fin-00121 Helsinki (FI).

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WO 01/00236 A1

(54) Title: PREVENTION OF TYPE 1 DIABETES AND OTHER NON-POLIO ENTEROVIRUS DISEASES

(57) Abstract: Live virus vaccines comprise attenuated viruses, while other vaccines comprise killed viruses or parts thereof. It has now been found that the immune response induced by oral poliovirus vaccine (OPV), which is a live vaccine, is cross-reactive with non-polio enteroviruses. OPV is therefore useful in the prevention of non-polio enterovirus diseases, especially Type 1 diabetes mellitus (IDDM). OPV is also useful in combination with killed/subunit non-polio enterovirus vaccines, whereby it prevents harmful side-effects of the killed/subunit vaccine by shifting the immune response from a harmful Th2- type response to a Th1 type response.

Prevention of Type 1 diabetes and other non-polio enterovirus diseases

Field of the Invention

The invention relates to the prevention of Type 1 diabetes and other non-polio enterovirus diseases by a novel vaccination regime based on extensive immunisations by currently available oral poliovirus vaccine (OPV) and/or by new non-polio enterovirus vaccines.

The invention provides prevention of Type 1 diabetes mellitus (IDDM) and other non-polio enterovirus diseases by eliminating the risk effect of enterovirus infections. This is achieved by a novel immunisation regime, which is based on the induction of systemic and local mucosal Th1-type T-cell immunity by oral poliovirus vaccinations and optionally induction of Th2-type humoral immunity by a new enterovirus vaccine which induces neutralizing antibodies against appropriate enterovirus serotypes. These two regimes can be used separately or in combination.

More precisely the present invention relates to the use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against non-polio enterovirus diseases, and especially against Type 1 diabetes mellitus (IDDM). When OPV is used together with a vaccine, which induces serotype specific immunity against non-polio enteroviruses, harmful side effects of the non-polio enterovirus vaccine can be avoided. The invention thus provides a vaccine composition comprising said two vaccines.

Background

Enterovirus infections are usually subclinical but cause also various kind of diseases. Typical enterovirus diseases are meningitis, paralysis, myocarditis, generalized infections in newborns, hand, foot and mouth -disease, herpangina, pleurodynia, hepatitis, rash, exanthemas and respiratory diseases including pneumonia. In addition, enterovirus infections have been suspected to play a role in the pathogenesis of dilated cardiomyopathy, atherosclerosis, postviral fatique syndrome and Type 1 diabetes mellitus.

The group of enteroviruses includes a total of 64 different serotypes. Polioviruses are the most widely known enteroviruses including 3 different serotypes (poliovirus types 1, 2 and 3) which all can cause meningitis and typical paralytic poliomyelitis (flaccid paralysis). Meningitis is frequently caused by several non-polio enteroviruses, which are the most common cause of aseptic meningitis. Myocarditis is caused mainly by coxsackie B serotypes but

also other enterovirus serotypes may be involved. Hand, foot and mouth - disease is mainly caused by certain coxsackie A serotypes and severe infections of infants are related to coxsackie B serotypes. Paralytic diseases can also be caused by some other serotypes than poliovirus serotypes. The serotypes related to atherosclerosis and Type 1 diabetes are not known. In type 1 diabetes the most suspected ones have been coxsackieviruses B4 and B5 but also other than coxsackie B serotypes may be involved.

The only enterovirus vaccine, which has been used in human beings is poliovirus vaccine. This vaccine includes all three poliovirus serotypes and gives effective prevention against paralytic poliomyelitis. The protection is based on the induction of neutralizing antibodies, against these serotypes and is serotype specific. Thus, neutralizing antibodies, which are induced by poliovirus vaccines do not protect against any other enterovirus serotypes than the three poliovirus serotypes. The role of T-cell mediated immune responses in the protection against poliovirus infections is not known. The generally accepted view is that they play only a minor role while antibodies are more important in the elimination of infection and in the protection against reinfections.

Two different types of poliovirus vaccine have been developed. The killed inactivated poliovirus vaccine (IPV; Salk vaccine) includes formalin-inactivated polioviruses (all 3 serotypes). This vaccine is given parenterally using subcutaneous injections. It induces a Th2-type immune response characterized by strong antibody response and high levels of neutralizing antibodies against all poliovirus serotypes and gives effective prevention against paralytic poliomyelitis. However, it induces only weak local immune response in the gut. As gut associated lymphoid tissue is the primary replication site of polioviruses, IPV vaccine can not protect against poliovirus infection but only against the complications of infections. IPV can induce only weak cytotoxic T-cell immune responses.

The other poliovirus vaccine is oral poliovirus vaccine (OPV; Sabin vaccine) which includes live attenuated polioviruses (all three serotypes). This vaccine is given *per os* and the virus replicates in the same way as the wild polioviruses in the body. As the vaccine is given *per os* in the same way as natural enterovirus infections are acquired, it induces strong local immunity in the intestine, which prevents from later poliovirus infections. Thus, OPV vaccinated individuals usually do not become infected by polioviruses because the

virus is not able to replicate in the intestine. The nature of this protection is not completely understood but it probably depends on both neutralizing antibodies and T-cell mediated immunity. OPV induces stronger T-cell responses than IPV and it induces mainly Th1-type T-cell responses characterized by strong cytotoxic T-cell responses.

Vaccines against non-polio enteroviruses are not available for human use. The reason is that the large number of enterovirus serotypes makes it difficult to make a pan-enterovirus vaccine and, on the other hand, the serotypes, which are causing the most severe non-polio enterovirus diseases, are highly variable. Myocarditis and cardiomyopathies have been associated with coxsackie B group viruses, meningitis and neonatal infections with several different serotypes and practically nothing is known about the serotypes possibly related to the development of atherosclerosis. In Type 1 diabetes the responsible serotypes are not known except that polioviruses are not involved. The general view is that poliovirus vaccines should not be effective in the prevention of Type 1 diabetes or other non-polio enterovirus diseases, but that the prevention of non-polio enterovirus diseases would require new vaccines which should induce neutralizing antibodies against the serotypes to be protected. Another reason for the lack of human non-polio enterovirus vaccines is that the safety of such vaccines has not been reliably confirmed. Thus, there is no effective vaccine or any other treatment for the prevention of non-polio enterovirus diseases in man.

Inactivated and subunit vaccines which include certain coxsackie B viruses have been tested in animal models. They have induced good antibody levels in mice and rabbits and effectively protected from infections caused by the serotypes which were included in the vaccine (Fohlman et al., 1990 and 1993; See and Tilles, 1994 and 1997). However, these vaccines have not been tested in human beings. The main reason for this is that the current knowledge on the mechanisms of immune protection against enteroviruses is limited and the safety of such vaccines can not be guaranteed. The safety issue has become very important after the discovery of the unexpected side-effects related to the use of inactivated respiratory syncytial virus (RSV) and measles vaccines in humans (Fulginiti et al., 1967; Harris et al., 1969; Kapikian et al., 1969). These vaccines paradoxically increased the severity or modulated the course of natural infections. The most probable explanation for these adverse effects is that these kind of inactivated vaccines generally in-

duce good antibody response but very poor cytotoxic T-cell response. Thus, they may have induced a shift towards Th2-type antibody mediated immunity which resulted in the atypical symptoms. This indicates the need for very detailed data on the effect of the vaccine on the course of natural infections and careful evaluation of the safety issues.

Another problem has been that the protection which is achieved by vaccines of this kind depends on the induction of neutralizing antibodies and the protection is therefore serotype specific. Accordingly, the vaccine should include the serotypes, which should be prevented. As described above, in non-polio enterovirus diseases the spectrum of responsible serotypes varies a lot from disease to disease and even in one disease like Type 1 diabetes the exact serotypes of responsible viruses have not yet been identified. Thus, the composition of the enterovirus serotypes to be protected is not known and may be different from one disease to another.

The advantage of the immunisation regime of the present invention is that it is based on the oral poliovirus vaccine (OPV) which has been extensively used in almost all countries of the world and which has proved to be very safe and effective. The poliovirus vaccines are actually one of the most effective and safest vaccines ever developed and have led to an almost complete eradication of poliovirus infections from the world. The only clinically relevant complication of OPV is the risk of vaccine associated paralysis. However, its frequency is extermely low (about 1 per 1-10 milj. vaccinees).

The general view is that immunity against enterovirus infection is based on the presence of neutralizing antibodies against the virus. These antibodies can efficiently neutralize the virus when it enters the body. The significance of neutralizing antibodies is reflected by the fact that patients who have abnormally low levels of antibodies due to an immune deficiency are particularly susceptible for enterovirus infections. Neutralizing antibodies can be detected for prolonged periods after the infection. They contribute to the eradication of the virus during primary enterovirus infection and protect against reinfections. However, they can not protect against infections, which are caused by other serotypes. Thus, the protection by these antibodies is serotype specific. Accordingly, it is generally thought that it is essensial for the efficacy of enterovirus vaccines that the vaccine is able to induce high titres of neutralizing antibodies against the serotypes which should be protected. The

only currently used enterovirus vaccine is poliovirus vaccine which includes all three poliovirus serotypes.

The present invention is based on the finding that, in contrast to the general paradigm, oral poliovirus vaccines could also protect against other enterovirus infections than poliovirus infections and could therefore be used for the prevention of various non-polio enterovirus diseases, which have been described in detail in previous paragraphs, and diseases where the role of enteroviruses has been suspected including Type 1 diabetes mellitus, chronic fatigue syndrome and atherosceloris. This protection would be based on efficient induction of T-cell responses and local mucosal immunity by repeated OPV vaccinations. T-cell immune responses are known to cross-react between certain enterovirus serotypes when analysed *in vitro* by T-cell proliferation assay (Beck and Tracy, 1990; Graham et al., 1993). However, it was not known whether this cross-reactivity had any biological significance *in vivo*. It was not either known to what extent T-cell responses which are induced by OPV vaccinations can cross-react with non-polio enteroviruses and whether this had any clinical relevance.

We have previously evaluated these questions by analysing enterovirus specific T-cell responses in young infants. We found that some infants, who had never experienced any coxsackievirus B infection according to the lack of neutralizing antibodies, had strong T-cell proliferation response against purified coxsackievirus B4 antigen, which probably reflects the cross-reactivity of T-cells which have initially been induced by other enterovirus infections (Juhela et al., 1998). In addition, polio vaccination at the age of 6 months induced stronger T-cell response to purified coxsackievirus B4 and poliovirus antigens in children who had serological evidence of previous enterovirus infection compared to children who had no previous enterovirus infections (Juhela et al., 1998). This suggests that T-cells can cross-react between polioviruses and non-polio enteroviruses.

Our aim is to utilise this T-cell cross-reactivity by priming cross-reactive T-cell memory using OPV vaccinations. This, in turn, would make the immune responses to other enteroviruses stronger and more rapid (secondary-type response) and in this way speed up the eradication of the virus during acute non-polio enterovirus infections. OPV can not totally protect from these infections as it does not induce neutralizing antibodies against non-polio enteroviruses but it may protect against viremia and severe illnesses by

potentiating the T-cell responses by inducing cross-reactive memory T-cells. This kind of T-cell help can potentiate both the production of neutralizing antibodies during infection as well as cytotoxic T-cell responses against non-polio enteroviruses. It may also booster antibodies against other enteroviruses than the serotype causing the acute infection by eliciting anamnestic immune responses. Induction of anamnestic responses means that OPV stimulates memory T-cell clones, which have originated from previous enterovirus exposures and in this way leads to their activation and induction of antibodies against all these serotypes. This kind of anamnestic response is used in the present regime to enhance enterovirus antibody levels in pregnant women thus providing protection for their infants.

Summary of the Invention

One object of the present invention is to provide a method of preventing non-polio enterovirus diseases, especially Type I diabetes (IDDM).

Another object of the invention is to provide a vaccine or vaccine composition useful in preventing said diseases.

Still another object of the present invention is to avoid harmful side effects of killed or subunit enterovirus vaccines that induce serotype specific immunity.

Still another object of the present invention is the use of a polio vaccine and/or a non-polio enterovirus vaccine in the manufacture of a vaccine against enterovirus diseases, especially Type I diabetes (IDDM).

The objects of the present invention are fulfilled by providing a method of preventing non-polio enterovirus diseases or of preventing Type 1 diabetes mellitus (IDDM) comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to a human subject.

The invention further encompasses the use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against non-polio enterovirus diseases, and especially for the manufacture of a vaccine against Type 1 diabetes mellitus (IDDM).

The invention is also directed to a vaccine composition comprising oral poliovirus vaccine (OPV) and a vaccine, which induces serotype specific immunity against non-polio enteroviruses. Preferably the non-polio enterovirus vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a coctail thereof.

The invention further relates to the use of a vaccine, which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16 for the manufacture of a vaccine against non-polio enterovirus diseases, especially Type 1 diabetes mellitus (IDDM). It also relates to said vaccine and to a method of preventing non-polio enterovirus diseases, especially IDDM, comprising administering an effective amount of said vaccine to a human subject.

The invention further provides a method of preventing non-polio enterovirus diseases, especially Type I diabetes mellitus (IDDM) in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to pregnant women, or comprising the administration of an effective amount of oral poliovirus vaccine (OPV) prenatally to the pregnant woman and postnatally to the baby.

A method of preventing non-polio enterovirus diseases, especially IDDM, comprising the administration of repeated doses of an effective amount of oral poliovirus vaccine (OPV) to children is provided.

Finally the invention encompasses a method of avoiding harmful side effects of non-polio enterovirus vaccines, which induce serotype specific immunity against non-polio enteroviruses comprising administering an effective amount of said non-polio enterovirus vaccine simultaneously, before or after administering an effective amount of oral poliovirus vaccine (OPV) to a human subject.

Brief Description of the Drawing

Figure 1 shows the cumulative prevalence of IDDM in cohorts which have never received OPV, or which have received one dose of OPV in childhood or *in utero*.

Detailed Description of the Invention

"OPV" is an abbreviation of oral poliovirus vaccine, and means a vaccine that comprises live attenuated polioviruses of one, two or all three of the serotypes or infectious cDNA or RNA thereof. Besides being administered orally OPV may also be given by any other mucosal route like *per rectum* or intranasally or it may be given parenterally. OPV comprising attenuated viruses of all three serotypes is commercially available and is also called Sabin vaccine.

"IPV" is another commercially available poliovaccine, which comprises killed inactivated polioviruses of all three serotypes. This vaccine is called Salk vaccine.

"IDDM" means insulin-dependent diabetes mellitus, which is the same as Type 1 Diabetes Mellitus or Type 1 Diabetes.

"Non-polio enterovirus diseases" means any disease caused by non-polio enteroviruses e.g. meningitis, paralysis, myocarditis, generalized infections in newborns, hand, foot and mouth -disease, herpangina, pleurodynia, hepatitis, rash, exanthemas, respiratory diseases including pneumonia, dilated cardiomyopathy, atherosclerosis, postviral fatigue syndrome and Type 1 diabetes mellitus.

"An effective amount" of a vaccine is an amount, which is able to elicit a protective immune response in the recipient, either by eliciting neutralizing antibodies or a cell-mediated response, or both.

A vaccine that induces "serotype specific immunity" is the same as a vaccine that induces neutralizing antibodies. Such vaccines may be killed vaccines, subunit vaccines or cDNA or RNA fragment vaccines, wherein the fragment encodes an antigenic part or an inactivated form of the virus.

A "killed vaccine" is the same as an inactivated vaccine i.e. a vaccine comprising viruses treated so that they have lost their infectivity. A "subunit vaccine" comprises only an antigenic part or parts of the viruses, not the whole viruses.

A "diabetogenic enterovirus" is an enterovirus that is associated with the induction of diabetes. These viruses are represented by the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16. However, also other serotypes of non-polio enteroviruses might be involved in the induction of diabetes.

Each of all three poliovirus serotypes can cause paralytic disease. Oral poliovirus vaccine used in the present invention may contain only one of them or their different combinations. Preferably it contains a cocktail of all these three serotypes (serotypes 1-3). The vaccine viruses of the OPV used are attenuated polioviruses, the virulence of which has been reduced. This may be carried out by different methods including serial passage of the virus in cell cultures, antigenic modification by chemical treatments, construction of recombinant or chimeric viruses, mutagenization of viral genome, deletion of

certain gene regions, selection of temperature sensitive mutants or irradiation. Alternatively, vaccine viruses may be attenuated natural poliovirus isolates or infectious poliovirus cDNA or RNA having reduced capability to cause clinical disease. Typically, the presented immunisation regime is based on the use of the commercially available and widely used Sabin oral poliovirus vaccine, which contains all three poliovirus serotypes in each vaccine dose. It is administered orally and replicates in the intestine, but does not cause paralytic polio or other clinical manifestations.

Each immunising dose of OPV includes infective viruses or infective RNA or cDNA in a titre, which is able to produce infection in humans. This dose would correspond to that which is used in the traditional Sabin-type live oral poliovirus vaccine including a minimum of $10^{5.5}$ - 10^{6} $TCID_{50}$ for poliovirus Type 1, 10^{5} $TCID_{50}$ for poliovirus type 2 and $10^{5.5}$ - $10^{5.8}$ $TCID_{50}$ for poliovirus type 3 live attenuated Sabin strains of polioviruses. The dose may also be another, if it has been confirmed to be safe and infectious. (TCID = tissue culture infectious dose; $TCID_{50}$= the dose which infects 50 % of the cultures.)

The new non-polio enterovirus vaccines of the present immunisation regime may include either whole viruses, the infectivity of which has been inactivated, or sub-unit vaccines containing certain antigenic structures of the virus, or their combination, or fragments of viral RNA or cDNA coding for antigenic structures of the virus. Inactivated vaccines may be produced by propagating the virus in cell cultures and by purifying it from infected cells and culture media by high-speed centrifugation in a density gradient formed by sucrose or other high-density media. Alternatively the virus could be purified by chromatography. The infectivity of the purified viruses is destroyed by inactivating the viruses by chemical treatment (e.g. formalin inactivation like that used to produce IPV), irradiation or heat treatment. Sub-unit vaccines may consist of purified viral proteins or recombinant viral proteins, synthetic peptides corresponding to viral antigenic epitopes or empty viral capsids, which are produced during infection but lack the viral genome. These subunit vaccines can be administered either as such or conjugated to haptens or carriers (e.g. ISCOM particles).

The new non-polio enterovirus vaccines can be given parenterally or by mucosal route like *per os*, *per rectum* or intranasally. Each immunising dose includes viral structures in a titre, which is able to induce proper immune response in humans. This dose would correspond to that used in Salk-type

inactivated poliovirus vaccine including 1.8 - 2 μg of viral protein per each dose and 20 - 40 antigenic D-units of poliovirus type 1, 4 - 8 antigenic D-units of poliovirus type 2 and 16 - 32 antigenic D-units of poliovirus type 3. The dose may also be another, if it has been confirmed to be safe and immunogenic.

In addition to the active ingredients that elicit an immune response, the OPV and the non-polio enterovirus vaccines used in the present invention may comprise pharmaceutically acceptable excipients, carriers, haptens and adjuvants. Excipients, carriers, haptens and adjuvants may include for example phenoxyethanol, magnesium chloride, sucrose, thiomersal, formaldehyde, phenol, antibiotics (preservatives) or aluminium salts, ISCOM particles, carrier proteins (e.g. cholera toxin), liposomes or protein micelles (haptens/adjuvants).

A new immunisation regime for the prevention of diseases caused by non-polio enteroviruses is introduced (Table 1).

Table 1. Main immunization regime

Action 1. *OPV during pregnancy*
Given preferentially during the first trimester but may also be given later during pregnancy. May also be given to women who are at fertile age but not pregnant.

Action 2. *OPV in childhood*
Given at the age of 0, 6, 10, 14 weeks, boosters at older age (e.g. every 5 years).

Action 3. *Killed/subunit vaccine*
Given at the age of 3, 6 and 12 months, boosters at older age. Can also be given to pregnant mothers.

Actions 1, 2 and 3 can be used separately or in different combinations. The timing of childhood OPV vaccinations in action 2 may vary but the first ones should preferentially be given by the age of 3 months.

Killed or subunit vaccine includes one or more of the following enterovirus serotypes or their antigenic structures: coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, coxsackievirus A serotypes 9 and 16. It can be given during pregnancy and at varying ages in childhood with booster given later in life. Killed or subunit enterovirus vaccine may be given simultaneously, before or after OPV is given.

Protection against non-polio enteroviruses is induced by extensive immunisation by repeated doses of live attenuated oral poliovirus vaccine (OPV). The regime includes prenatal and postnatal OPV vaccinations, which can be used in combination or separately. Prenatal vaccination is carried out by giving OPV to pregnant women in order to protect the child *in utero* and in infancy (Action 1 in Table 1). This protection is based on anamnestic immune response, which is induced by OPV-vaccination. Anamnestic response is due

to the activation of cross-reactive enterovirus specific memory T-cell clones and leads to increases in antibody levels against those enterovirus serotypes to which the mother has been exposed prior to the OPV vaccination. Anamnestic antibody response of the mother protects the child because IgG class maternal antibodies are transferred to the fetus through the placenta and are thus protecting the child until the age of 6-12 months when maternal antibodies disappear from child´s circulation.

Postnatal vaccination (Action 2) is carried out like OPV vaccination shedules in general but may be more extensive to get maximal stimulation of cross-reactive T-cell immunity (Table 1). It includes repeated vaccinations, first ones given at birth and during the first weeks of life followed by booster vaccinations in childhood with a few years intervals (like in WHO EPI-program). OPV-vaccination *per os* induces also strong local immune response in mucosal surfaces, particularly in the gut. This is important because the primary replication site of enteroviruses is gut-associated lymphoid tissue. This local immunity is targeted also to non-polio enteroviruses because of OPV induced cross-reactive T-cell response and induction of local production of interferons.

The Actions 1 and 2 of this regime can be combined with new non-polio enterovirus vaccines, which induce serotype specific immunity to get maximal protective effect against non-polio enteroviruses (Action 3 in Table 1). Serotype specific immunity may be induced by killed enterovirus particles or sub-unit vaccines carrying certain enterovirus structures or peptides. This serotype specific vaccine can be given to pregnant mothers as well as to children as described in Table 1. The serotype specific vaccine preferably includes one or more of the following enterovirus serotypes (coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16). This kind of killed or subunit vaccines induce efficient antibody response but the protection is specific for those viruses which are included in the vaccine (protection by neutralizing antibodies is serotype specific). In such combination OPV can be used to give additional protection by cross-reactive T-cell responses against the serotypes which are not included in the killed/subunit vaccine. OPV can also be used to booster the antibody responses which are induced by killed/subunit vaccines. OPV can also be used to direct the immune responses induced by killed/subunit vaccines to Th1-type responses rather than Th2-type responses. Th2-type responses are typically induced by killed/subunit vaccines and can be associ-

ated with serious side-effects leading to more severe course of natural infection in vaccinated individuals (like observed in individuals vaccinated by killed RSV or measles vaccines). OPV like other live vaccines induces mainly Th1-type responses leading to cytotoxic T-cell responses and can thus counteract the Th2-type responses induced by killed/subunit vaccines by inducing cross-reactive Th1 -type T-cells. To avoid Th2-responses OPV may be given either before, simultaneously with or after the killed/subunit vaccines are given.

Thus, the present regime includes OPV-vaccinations to induce systemic T-cell responses and local mucosal immunity as well as anamnestic antibody responses in pregnant mothers (Actions 1 and 2). OPV vaccinations can be combined with new inactivated or subunit enterovirus vaccines (Action 3). This combination would give maximal preventive effect (neutralizing antibodies induced by killed/ subunit vaccines are the first barriers against infections and T-cell immunity induced by OPV helps in the eradication of infection). OPV may also be used in combination with inactivated or subunit vaccines to prime or booster their effect or to prevent possible harmful side-effects caused by Th2-type bias in immune response to enteroviruses which may be caused by inactivated or subunit vaccines.

We have found that there are unexpected side-effects of IPV vaccines, which increase the risk of complications of non-polio enterovirus infections like Type 1 diabetes by directing the immune response against non-polio enteroviruses into the Th2 direction. However, OPV is benefical, because it decreases the risk of complications of non-polio enterovirus infections and vaccinations of inactivated/subunit non-polio enterovirus vaccines (e.g against diabetes) by inducing cross-reactive memory T-cells, by directing the immune response to non-polio enteroviruses into the Th1 direction and by inducing local protection in the mucosal tissues.

One advantage of this invention is that it is based on a widely used and very safe vaccine (OPV) but gives a new indication for this vaccine, which has not been previously suggested. The novel aspects are also that the invention utilises strong T-cell responses induced by live OPV vaccine, the cross-reactivity of these responses between different enterovirus serotypes, induction of local immune responses by OPV in mucosal surfaces in pharynx and in gut, vaccination of both pregnant women and children, and optional combination of OPV and new serotype specific killed/subunit vaccines to booster their effect and to avoid their side-effects related to Th2-based re-

sponses. An additional novel aspect is that the inactivated/subunit vaccine includes serotypes, which are the most important in the pathogenesis of severe non-polio enterovirus diseases including Type 1 diabetes.

This vaccination regime can be used in the whole population or in specific high-risk groups such as children with genetic risk alleles for Type 1 diabetes, children with diabetes in first-degree relatives or children positive for diabetes-related autoantibodies.

This vaccination regime is the only possibility which is currently available for the prevention of non-polio enterovirus diseases in man. It can be implemented into clinical work immediately as it is based on currently widely used and well-tolerated vaccine (OPV). It can be coupled with inactivated or subunit enterovirus vaccines to increase their preventive effect and to avoid their side-effects.

In Finland practically the whole population was vaccinated by one dose of OPV in February - March in the year 1985 to eradicate the last polioepidemic (Hovi et al., 1986; Harjulehto-Mervaala et al., 1994). This provides an excellent possibility to analyze possible effects of OPV vaccination on the risk of type 1 diabetes because IPV has been used as the only poliovirus vaccine for decades and has also been used after the epidemic. OPV vaccination was also given to pregnant women (Harjulehto-Mervaala et al., 1993). We have analysed the cumulative prevalence of type 1 diabetes in birth cohorts which have received OPV vaccination in the year 1985 either in childhood or *in utero* and compared that to cumulative prevalence in birth cohorts who had never received OPV (Figure 1). The Figure shows the cumulative prevalence of Type 1 diabetes (IDDM) per 100,000 children by the age of 8 years in Finland in birth cohorts which have either never received oral poliovirus vaccine (OPV) or have been vaccinated by one dose of OPV in childhood or *in utero* during the mass-vaccination campaign in 1985. The cumulative prevalence of type 1 diabetes was significantly lower in OPV-vaccinated cohorts compared to unvaccinated cohorts: The average prevalence in OPV vaccinated cohorts born in the years 1980-1985 was 272 compared to 326 in unvaccinated cohorts born in 1986-1989 ($p<0.01$ in student´s t-test). The prevalence of diabetes was also low in children whose mother had been vaccinated during pregnancy (261 per 100,000; Figure 1). These findings indicate that both Actions 1 and 2 in the proposed immunisation regime (see Table 1) have a protective effect against Type 1 diabetes.

We have also found that incidence of Type 1 diabetes correlates with the type of poliovirus vaccine used in different countries. This correlation is not absolute but there is a general tendency to a lower incidence of Type 1 diabetes in countries where OPV is used compared to countries where inactivated (killed) poliovirus vaccine (IPV) is used. In Finland the incidence of Type 1 diabetes is the highest in the world, and Finland is also one of the very few countries where IPV has been used as the only poliovirus vaccine for several decades (except in the year 1985 as mentioned before).

A possible role of different poliovaccination regimes as a cause of the international differences in the incidence of Type 1 diabetes is also supported by our findings in Estonian and Finnish children. In Estonia, where the incidence of Type 1 diabetes is one third of that in the neighbouring Finland, OPV is used as the only poliovirus vaccine in contrast to Finland where IPV is used. We analysed T-cell proliferation responses to tetanus toxoid, poliovirus type 1, coxsackievirus B4 (CBV4) and adenovirus antigens in 9-months-old infants in both countries. The responses to poliovirus and CBV4 were significantly higher in Estonian than in Finnish children ($p<0.05$) while responses to other antigens did not differ between the groups. Neutralizing antibodies against CBV group enteroviruses did not differ between the groups suggesting that the observed difference in T-cell responses was not due to different exposure of infants to enteroviruses in the two countries. Accordingly, the higher T-cell response to purified CBV4 virus in Estonian children probably reflects cross-reactivity of T-cells primed by previous OPV vaccinations. In Finland, the IPV vaccine is used which does not induce as high T-cell responses as OPV and which is also given at older age than OPV in Estonia (Estonian children had received three doses of OPV compared to one dose of IPV in Finnish children by the age of 9 months). This suggests that the OPV vaccination schedule in Estonia induces stronger cross-reactive immune response to non-polio enteroviruses than the IPV vaccination schedule used in Finland. This indicates that Action 2 in our immunisation regime (see Table 1) has a protective effect against Type 1 diabetes.

In the Finnish Diabetes Prediction and Prevention study (DIPP) we have analysed the frequency and serotype of enterovirus infections in 21 infants who have been followed from the birth and who have manifested with clinical Type 1 diabetes or turned positive for diabetes-related autoantibodies as a marker of subclinical beta-cell damage. Enterovirus infections were more

frequent in these children than in 104 control children matched for the time of birth, gender and HLA-risk alleles for Type 1 diabetes ($p<0.03$). This difference was particularly clear in infections which occurred 0-6 months before autoantibodies appeared: 57% of autoantibody positive subjects had an enterovirus infection during that period compared to 31% of control subjects of the same age (OR 3.7, 95% CI 1.2-11.4) (unpublished observation). During this period 29% of autoantibody positive children were positive for enterovirus RNA in serum compared to 6% of control subjects (OR 8.4, 95% CI 1.7-40.2). The results suggest that enterovirus infections are important risk factors for Type 1 diabetes and able to initiate the beta-cell damaging process in genetically susceptible individuals. The average age of the infants at the appearance of autoantibodies was 9 months suggesting that diabetogenic enterovirus infections may occur already during the very first months of life.

The serotype of enterovirus infections related to induction of autoantibodies or manifestation of clinical diabetes has been analysed in the DIPP study and in the previous Childhood Diabetes in Finland (DiMe) study. These serotypes are included in the killed/subunit vaccine in the present immunisation regime (Action 3 in Table 1).

OPV vaccinations can be combined not only with serotype specific vaccines but also with passive immunisation regimes against enteroviruses. This kind of passive immunisation may include e.g. immunoglobulins which contain enterovirus specific antibodies and which are given intravenously or orally.

Example 1

We have analysed the effect of OPV vaccination on the course of subsequent coxsackievirus B3 (CBV3) infection in mice. In these studies we used a transgenic BALB/c strain which expresses human poliovirus receptor and can therefore be infected by human polioviruses (Horie et al., 1994).

Transgenic BALB/c mice were first immunized by live poliovirus vaccine (Sabin strain of poliovirus type 1) or inactivated poliovirus vaccine IPV, and later challenged to a pancreas-tropic strain of coxsackievirus B3 (Nancy strain). (IPV was the commercially available poliovirus vaccine Novum purchased by National Public Health Institute of Holland). Two doses of live poliovirus vaccine strain type 1 (Sabin) were given intramuscularly with two weeks intervals (10^6 $TCID_{50}$/mouse, first injection at the age of 8 weeks). Two doses of killed poliovirus vaccine were given intramuscularly in the same way (0.1 μg

per mouse). Two weeks after the last poliovirus injection the mice were infected by coxsackievirus B3. T-cell proliferation responses were analysed two weeks after the coxsackievirus B3 challenge using standard blast-transformation test and highly purified viruses as antigens. The T-cell responses are expressed as spesific counts (mean cpm), and and the results are shown in Table 2.

Table 2. Effect of previous poliovirus immunisation on T-cell proliferation responses during subsequent coxsackievirus B3 infection in transgenic mice expressing human poliovirus receptor.

	Proliferation response in different immunisation groups (mean cpm values)		
Virus antigen	PBS (N=5)	IPV (N=5)	Sabin (N=5)
Coxsackievirus B3	1444	3669	6485
Poliovirus type 1	1927	4898	6738

Grading of coxsackievirus B3 induced pancreatitis and myocarditis as well as the detection of viremia was done two weeks after the coxsackievirus B3 challenge. The presence of viremia was analysed at the same time by detecting viral RNA in serum using a sensitive RT-PCR method. The results are shown in Table 3.

Table 3. Effect of previous poliovirus immunisation on the pathogenesis of subsequent coxsackievirus B3 infection in transgenic mice expressing human poliovirus receptor.

		Immunisation group		
		PBS (N=5)	IPV (N=5)	Sabin (N=5)
Pancreatitis	+ + +	2	0	4
	+ +	3	2	1
	+	0	1	0
	-	0	2	0
Myocarditis	+ + +	0	0	0
	+ +	0	0	1
	+	3	2	3
	-	2	3	1
Viremia	+	2	3	1
	-	3	2	4

In the experiments live poliovirus vaccine (Sabin strain) increased *in vitro* T-cell proliferation responses during subsequent coxsackievirus B3 infection. This increase was observed in proliferation responses against both purified coxsackievirus B3 and poliovirus type 1 (Table 2). This suggests that previous live poliovirus vaccination can augment cellular immune responses during subsequent non-polio enterovirus infection. Previous IPV vaccination also enhanced T-cell responses during subsequent coxsackievirus B3 infection but the effect was weaker that that of live vaccine (Table 2).

Previous immunisation with live poliovirus vaccine (poliovirus type 1, Sabin vaccine strain) increased T-cell infiltration in the pancreas during sub-

sequent infection with a pancreas-tropic strain of coxsackievirus B3 (Table 3). In contrast, previous IPV vaccination was associated with weak T-cell infiltration in the pancreas as compared to that observed after live poliovirus vaccine or that in control mice. These results suggest that live poliovirus vaccination augment *in vivo* T-cell responses during subsequent non-polio enterovirus infections while killed poliovirus vaccine may have an opposite effect.

There was also a tendency of a low frequency of viremia in mice previously immunised with live poliovirus. This suggests that live poliovirus vaccination facilitates the eradication of subsequent non-polio enterovirus infections.

In another experiment it was found that altogether 9 (92%) out of the twelve poliovaccinated mice had T-cell infiltration in the heart compared to 7 (53%) of the fifteen unvaccinated mice. This suggests that prior challenge by live poliovirus exaggerates T-cell response during CBV3 infection *in vivo*.

Example 2

We have produced and tested formalin-inactivated coxsackievirus B vaccines in mice. These vaccines were produced by inactivating sucrose gradient purified viruses by 14 days incubation at +37 °C in 0.01% formalin in PBS.

Mean IgG1 antibody levels against purified coxsackievirus B3 were determined in Balb/c mice immunized by 3 repeated intramuscular injections with formalin-inactivated coxsackievirus B3 vaccine or phosphate buffered saline (PBS). Injections were given with two weeks intervals (first one at 8 weeks of age) and antibodies were measured at 2 weeks after the last vaccination. Antibody levels are expressed as OD_{492} values in EIA (Table 4).

Table 4. Antibody levels induced by inactivated coxsackievirus B3 vaccine in mice

	Immunization group	
Serum dilution	PBS (N=5)	Coxsackievirus B3 vaccine (N=5)
1/1600	0.12	0.95
1/6400	0.13	0.47
1/25600	0.14	0.28

Presence of viremia (virus in serum) was determined in BALB/c mice immunized with three repeated intramuscular injections with formalin-inactivated coxsackievirus B3 vaccine or with phosphate buffered saline (PBS) and subsequently infected with a pancreas-tropic strain of coxsackievirus B3 (Nancy strain, 10^6 $TCID_{50}$/mouse). Immunisations were done with two weeks intervals (first one at 8 weeks of age) and mice were infected 2 weeks after the last injection. The presence of virus in serum (viremia) was analysed three days after the infection using the end-point dilution assay of infectivity. End-point dilution of infectivity in LLC-cell cultures is presented in Table 5.

Table 5. Protection against viremia by immunisation with an inactivated coxsackievirus B3 vaccine

	Immunisation group	
Mice	PBS	Coxsackievirus B3 vaccine
1.	10^{-3}	ND
2.	10^{-3}	ND
3.	10^{-1}	ND
4.	10^{-4}	ND
5.	10^{-1}	ND

ND: Not detectable (titre $<10^{-1}$)

As shown in Table 4 immunisation with inactivated coxsackievirus B3 vaccine induced high levels of antibodies as measured against purified coxsackievirus B3 in EIA test. We also found that vaccination completely protected the mice against infection by a pancreas-tropic strain of coxsackievirus B3. Virus could not be detected in the serum in any of the vaccinated animals while all control mice were positive for the virus (Table 5). This vaccine also protected the mice from virus-induced pancreatitis: None of the vaccinated animals had T-cell infiltration in the pancreas while all control mice had a very strong inflammatory response.

These results suggest that inactivated non-polio enterovirus vaccines are effective in the protection against non-polio enterovirus infections. This protection is probably mediated by neutralizing antibodies induced by the vaccine.

Example 3

SJL/J mice were first immunised either with formalin-inactivated poliovirus vaccine (IPV; 0.1 μg/mouse), or with saline (PBS). After 14 days the mice were infected with coxsackievirus B3 intramuscularly (10^6 $TCID_{50}$/mouse).

Histopathology of the pancreas was analysed 14 days after the infection. The results are shown in Table 6.

Table 6. Inflammation reaction (T-cell infiltration) in the pancreas of SJL/J mice infected intramuscularly with a pancreas tropic strain of coxsackievirus B3 (Nancy strain).

	Vaccine	
Pancreatic inflammation	PBS (N=5)	IPV (N=5)
Strong	1	4
Moderate	2	1
Not detected	2	0

Our observations indicate that IPV increases the severity of non-polio enterovirus infections. We have found that mice, which have first been immunized by IPV and later infected with a non-polio enterovirus, namely a pancreas tropic Nancy strain of coxsackievirus B3, had more severe pancreatitis than mice which had not previously been immunised with IPV (Table 6).

Mean IgG1 antibody levels against purified coxsackievirus B3 were determined in BALB/c mice immunized with three intramuscular injections with formalin-inactivated poliovirus vaccine (IPV; 0.1 μg per mouse) or with phosphate buffered saline (PBS) and subsequently infected with a pancreas-tropic strain of coxsackivirus B3 (Nancy strain, 10^6 $TCID_{50}$/mouse). Immunisations were done with two weeks intervals (first one at 8 weeks of age), mice were infected 2 weeks after the last injection and antibodies were measured 2 weeks after the infection. Antibody levels are expressed as mean OD_{492} values in EIA (Table 7). IPV was the commercially available poliovirus vaccine Novum purchased by National Public Health Institute of Holland.

Table 7. Effect of immunisation with inactivated poliovirus vaccine on antibody response during subsequent coxsackievirus B3 infection

	Immunisation group	
Serum dilution	PBS (N=5)	IPV (N=5)
1/1600	0.50	0.37
1/6400	0.36	0.11
1/25600	0.32	0.08

IPV vaccination was associated with abnormally low antibody response during subsequent coxsackievirus B3 infection in BALB/c mice (Table 7). This suggests that immunisation with killed poliovirus vaccine may weaken antibody responses during subsequent non-polio enterovirus infections *in vivo*. This, in turn, may increase the severity of non-polio enterovirus infections.

We assume that the harmful effect of IPV is due to its ability to induce Th2-type immune responses. It has been shown previously that inactivated vaccines induce mainly Th2 type responses and that this kind of Th2 bias may increase the severity of natural infections (like in the case of inactivated respiratory syncytial virus and measles vaccine). This harmful effect is manifested particularly in infections caused by other serotypes than that used in the vaccine while infections by the same serotype as that used in the vaccine are totally protected by the vaccine (as shown in our mice experiments described in Table 5). This serotype-specific protection is based on vaccine-induced neutralizing antibodies. Thus IPV vaccination in childhood primes poliovirus specific immune response towards Th2 direction, which imprints T-cell memory in later enterovirus infections. Due to cross-reactive T-cells this Th2-bias will spread to immune responses against non-polio enteroviruses thus increasing the severity of non-polio enterovirus infections and the risk of their complications like Type 1 diabetes.

In contrast to the harmful effect of IPV on immune protection against non-polio enterovirus infections, OPV has a beneficial effect. As a live vaccine OPV induces stronger T-cell responses than IPV. In addition, this immune response is more balanced resembling that observed in natural enterovirus infections including both Th1- and Th2-type immune responses. This response is targeted to both structural and non-structural virus proteins, while IPV induces only response to structural virus proteins. By inducing strong T-cell responses OPV activates also memory T-cells, which can cross-react between polio and non-polio enteroviruses and booster both T-cell and antibody responses against non-polio enteroviruses. By this mechanism, OPV facilitates the clearance of non-polio enterovirus infections thus preventing from their complications. Thus, the risk of complications of non-polio enterovirus infections (like Type 1 diabetes) can be prevented by OPV.

In addition to the natural non-polio enterovirus infections, OPV can also be used to convert immune responses, which have been induced by inactivated or sub-unit enterovirus vaccines from Th2-type responses to Th1 direction. In this way OPV can be used to protect from the Th2-dependent side effects of inactivated or sub-unit non-polio enterovirus vaccines. This kind of side effects have been described in the context of the use of inactivated respiratory synsytial virus and measles virus vaccines and they include abnormal course of infections, increased severity of the infection, increased risk of complications of the infection and possible development of allergies and asthma.

Accordingly, OPV can be used to dictate the immune response induced by inactivated or subunit enterovirus vaccines to Th1-type responses thus protecting against the side-effects of such vaccines. In contrast, IPV may have an opposite effect increasing the risk of complications of non-polio enterovirus infections by dictating the immune response to Th2 direction.

An additional advantage of OPV over IPV is that as a live virus it induces production of interferon-alpha. It is induced only during virus infections and is the most potent antiviral cytokine (part of the innate immunity). It specifically protects against virus infections and provides protection before the antigen specific immune responses are induced. As a live virus OPV induces interferon-alpha and this induction happens both in mucosal surfaces and systemically. Vaccine viruses replicate in the gut for several weeks, which means that local production of interferon-alpha persists for prolonged periods in child-

ren repeatedly vaccinated by OPV. This will augment to the protective effect of OPV against non-polio enterovirus infections.

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We claim:

1. Use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against non-polio enterovirus diseases.

2. Use of oral poliovirus vaccine (OPV) for the manufacture of a vaccine against Type 1 diabetes mellitus (IDDM).

3. Use according to claim 1 or 2 for the manufacture of a vaccine to be administered in repeated doses to children.

4. Use according to claim 3 for the manufacture of a vaccine to be administered by the age of 3 months.

5. Use according to claim 4 for the manufacture of a vaccine to be administered at the age of about 0, 6, 10, and 14 weeks and boosters at older age.

6. Use according to claim 1 or 2 for the manufacture of a vaccine to be administered to pregnant women to protect their offspring against said diseases.

7. Use according to claim 6 for the manufacture of a vaccine to be administered prenatally to the pregnant woman and postnatally to the baby.

8. Use according to any of claims 1 to 7 for the manufacture of a vaccine to be administered in combination with a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

9. Use according to claim 8 wherein said serotype specific immunity inducing vaccine is a killed enterovirus vaccine or a subunit vaccine.

10. Use according to claim 8 wherein said serotype specific immunity inducing vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a cocktail thereof.

11. Use according to claim 8 wherein said serotype specific immunity inducing vaccine is a vaccine against one or more serotypes selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

12. A vaccine composition comprising oral poliovirus vaccine (OPV) and a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

13. The vaccine composition according to claim 12 wherein said serotype specific immunity inducing vaccine is a killed enterovirus vaccine or a subunit vaccine.

14. The vaccine composition according to claim 12 wherein said serotype specific immunity inducing vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a cocktail thereof.

15. The vaccine composition according to claim 14 wherein said serotype specific immunity inducing vaccine is a vaccine against one or more serotypes selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

16. Use of a vaccine, which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16 for the manufacture of a vaccine against non-polio enterovirus diseases, especially Type 1 diabetes mellitus (IDDM).

17. Use according to claim 16 for the manufacture of a vaccine to be administered to pregnant women or children.

18. Use according to claim 16 for the manufacture of a vaccine to be administered prenatally to the pregnant woman and postnatally to the baby.

19. A method of preventing non-polio enterovirus diseases comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to a human subject.

20. A method of preventing Type 1 diabetes mellitus (IDDM) comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to a human subject.

21. A method of preventing non-polio enterovirus diseases in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to pregnant women.

22. A method of preventing Type 1 diabetes mellitus (IDDM) in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) to pregnant women.

23. A method of preventing non-polio enterovirus diseases, especially IDDM, comprising the administration of repeated doses of an effective amount of oral poliovirus vaccine (OPV) to children.

24. The method of claim 23 wherein the first OPV is administered by the age of 3 months.

25. The method of claim 24, wherein the OPV is administered at the age of about 0, 6, 10, and 14 weeks and boosters at older age.

26. A method of preventing non-polio enterovirus diseases, especially IDDM, in the offspring comprising the administration of an effective amount of oral poliovirus vaccine (OPV) prenatally to the pregnant woman and postnatally to the baby.

27. The method of any of claims 19 to 26, wherein the administration of OPV is combined with the administration of a vaccine, which induces serotype specific immunity against non-polio enteroviruses.

28. The method of claim 27 wherein the serotype specific immunity inducing vaccine is a killed enterovirus vaccine or a subunit vaccine.

29. The method of claim 27 wherein the serotype specific immunity inducing vaccine comprises enterovirus antigens representing diabetogenic enterovirus serotypes or a cocktail thereof.

30. The method of claim 29 wherein the serotype specific immunity inducing vaccine is a vaccine against one or more serotypes selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

31. A method of preventing non-polio enterovirus diseases, especially IDDM, comprising administering an effective amount of a vaccine, which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

32. The method of claim 31 wherein the vaccine is administered to pregnant women or children.

33. The method of claim 31 for preventing the disease in the offspring comprising the administration of the vaccine prenatally to the pregnant woman and postnatally to the baby.

34. A vaccine which induces serotype specific immunity against one or more serotypes of diabetogenic non-polio enteroviruses selected from the group consisting of coxsackievirus B serotypes 1, 2, 3, 4, 5 and 6, echovirus serotypes 3, 4, 6, 9, 11, 22 and 30, and coxsackievirus A serotypes 9 and 16.

35. A method of avoiding harmful side effects of non-polio enterovirus vaccines, which induce serotype specific immunity against non-polio enteroiruses, said method comprising administering an effective amount of said non-polio enterovirus vaccine simultaneously, before or after administering an effective amount of oral poliovirus vaccine (OPV) to a human subject.

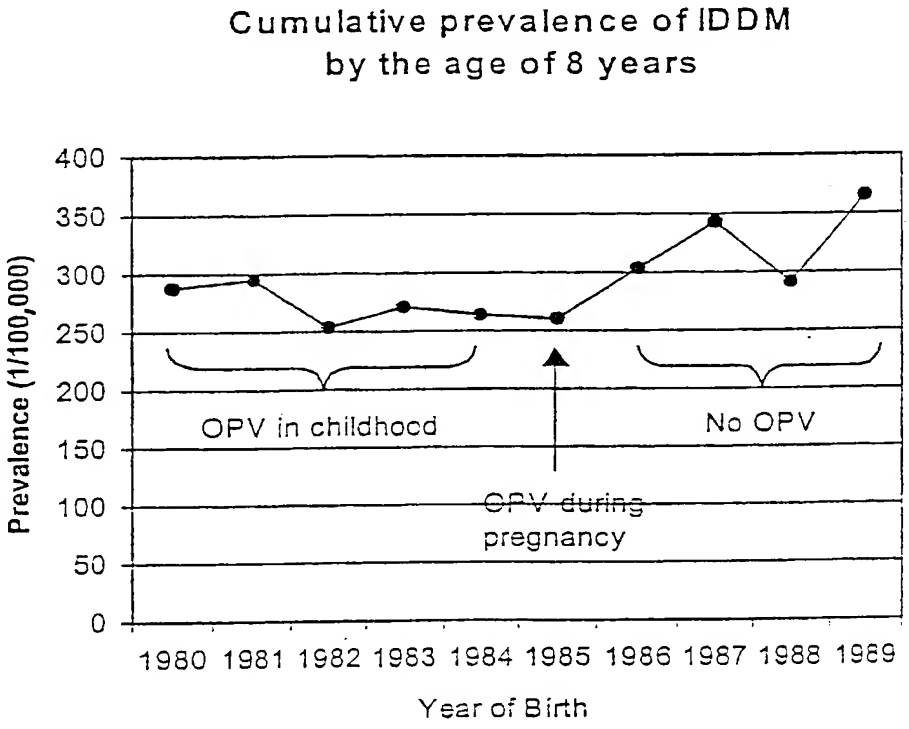
Cumulative prevalence of IDDM
by the age of 8 years
Prevalence (1/100,000)
400
350
300
250
200
150
100
50
0
OPV in childhood
No OPV
OPV during
pregnancy
1980 1981 1982 1983 1984 1985 1986 1987 1988 1989
Year of Birth

Figure 1

INTERNATIONAL SEARCH REPORT

International Application No
PCT/FI 00/00220

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/125

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILTUNEN M ET AL: "Immunisation and Type 1 Diabetes Mellitus" DRUG SAFETY, vol. 20, no. 3, 1999, pages 207-212, XP002901112 the whole document ---	1-35
X	US 5 723 283 A (CLASSEN JOHN BARTHELOW) 3 March 1998 (1998-03-03) column 2, line 62 -column 3, line 8 ---	1-7, 19-26
A	FOHLMAN J ET AL: "Vaccination of Balb/c mice against enteroviral mediated myocarditis" VACCINE, vol. 8, August 1990 (1990-08), pages 381-384, XP002901113 the whole document ---	1-35

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 July 2000	25 AUGUST 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	H. Nilsson

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE DIALOG INF. SERV. MEDLINE [Online] CLEMENTS G B ET AL: "Coxackie b virus infection and onset of childhood diabetes comments" retrieved from MEDLINE, FILE 155, accession no. 08341526 Database accession no. 95341923 XP002901114 abstract & LANCET (ENGLAND), 2 September 1995 (1995-09-02), pages 221-223, 346 (8969) ISSN: 0140-6736 ---	1-35
A	JUHELA S ET AL: "Enterovirus Infections and Enterovirus Specific T-Cell Responses in Infancy" J. MED. VIROLOGY, vol. 54, 1998, pages 226-232, XP002901115 introduction page 229, right-hand column -page 230, left-hand column -----	1-35

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/FI 00/00220

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5723283 A	03-03-1998	US 5728385 A	17-03-1998
		AU 7630094 A	14-03-1995
		CA 2169289 A	23-02-1995
		EP 0716612 A	19-06-1996
		WO 9505193 A	23-02-1995

INTERNATIONAL SEARCH REPORT

International application No.
FI00/00220

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 19-33,35
because they relate to subject matter not required to be searched by this Authority, namely:

see next sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
FI00/00220

Claims 19-33, 35 relate to methods of treatment of the human or animal body by surgery or by therapy/ diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.